

UNCLASSIFIED

AD NUMBER	
AD004669	
CLASSIFICATION CHANGES	
TO:	unclassified
FROM:	restricted
LIMITATION CHANGES	
TO: Approved for public release; distribution is unlimited.	
FROM: Distribution authorized to DoD only; Administrative/Operational Use; 01 APR 1952. Other requests shall be referred to Chemical Corps, Army Chemical Center, MD. Pre-dates formal DoD distribution statements. Treat as DoD only.	
AUTHORITY	
E.O. 10501 dtd 5 Nov 1953; BDRL ltr dtd 24 Nov 1971	

THIS PAGE IS UNCLASSIFIED

UNCLASSIFIED

AD _____

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION ALEXANDRIA, VIRGINIA

DOWNGRADED AT 3 YEAR INTERVALS:
DECLASSIFIED AFTER 12 YEARS
DCD DIR 5200.10



UNCLASSIFIED

THIS REPORT HAS BEEN DECLASSIFIED
AND CLEARED FOR PUBLIC RELEASE.

DISTRIBUTION A
APPROVED FOR PUBLIC RELEASE;
DISTRIBUTION UNLIMITED.

SECURITY INFORMATION

RESTRICTED

WAR DEPARTMENT
PHYSICAL SCIENCES DIVISION
CHEMICAL CORPS BIOLOGICAL LABORATORIES

Project no. 4-61-14-001

Report No. 22

1 December, 1951 to 1 April, 1952

Project on Marine Biology

DA-18-064-CML-471

K. F. Meyer, M. D.

Responsible Investigator

Robert Mills

Senior Laboratory Technician to Feb. 1, 1952

Lucile E. Foster

Volunteer on Plankton Work

Shelly A. Byers

Graduate Research Bacteriologist from 1 Feb., 1952

James M. Lucas

Laboratory Technician from 1 Feb 1952

Mary C. Edwards

Confidential Secretary (Part time)

University of California
The George Williams Hooper Foundation
San Francisco, California

SECURITY INFORMATION

AD No. 4669
ASTIA FILE COPY

SECURITY INFORMATION

1.

RESTRICTED

Plankton Studies

Field

During this period no routine sampling was done. The only collection trips were for sea water and tideland mud for the media.

Laboratory

Further studies were made on a better method for aerating the 20-liter bottles.

Method 4 is a direct aeration by bubbling air into the media from an aquarium pump. The tiny bubbles rising to the surface from the glass filter proved too disturbing and numbers of *Gonyaulax* died.

Method 5 is the same direct method but protecting the volume of the media by a glass sleeve over the aeration tube. This proved more satisfactory. Although this method gives more satisfactory results, the larger bottles still do not equal the test tube cultures.



Having a constant water bath temperature $12^{\circ} - 16^{\circ} \text{C}$ and a constant aeration, as Method 5, the next step is the standardization of the media, such as an artificial sea water media.

RESTRICTED

SECURITY INFORMATION

2.

Extraction methods

With small volumes the routine has been "X" method--centrifuge culture, decant the media, add equal volume of 0.1N HCl, boil for 10 minutes. All the test tube cultures are tested in this manner.

When larger volumes were used it was necessary to find a rapid method of concentrating the Gonyaulax. The following methods were tried:

"O". Seintered glass plate, filtration--too slow and plate easily clogged

"Y" Celite column, washed with distilled H₂O, culture filtered by vacuum. The Celite and Gonyaulax extracted by boiling 10 minutes with 50% Ethanol + 1 m/liter of conc HCl.

Two other methods have been tried in the attempt to find a better extraction method.

When the culture was put through air-driven Sharples at 50,000 rpm at the rate of 100-200 ml/minute, the procedure was very easy but the loss in poison when scraping the Sharples bowl was great.

The extraction of large volumes was best effected by vacuum filtration through a large surface, small volume of Celite No. 512 filter. The poison is then eluted without vacuum with 50% acidified ethanol as a part of the first orange-colored fraction. For example, Experiment No 2: the Gonyaulax were filtered out by the preceding method. The poison is then eluted with 50% acid ethanol in the orange-colored fraction.

SECURITY INFORMATION

SECURITY INFORMATION

RESTRICTED

Eluate No. 1	orange pigment	238 ml	25 Mu/ml
No. 2	yellow	100 ml	<10 Mu/ml
No. 3	green	100 ml	<10 Mu/ml
No. 4	green	100 ml	<10 Mu/ml

The majority of the poison comes out in the first eluate, but in order to be sure if this is the best method it is to be run in parallel with the direct method X

The test tube cultures in the small tank showed a high of 12,200 dinoflagellates per ml in media No. 6, while the best poison of 0.55 ml/Mu was produced by 3,370 Gonyaulax for a mouse unit. In February the bottom of the small tank rusted out and was sent out for repairs. Small flasks were used in the large tank.

During the period the best growth in the large bottles was with media No. 6 - 7,900 Gonyaulax per ml. The best poison was 1.8 ml/Mu or 5,807 Gonyaulax to make a mouse unit.

K. J. Megley

SECURITY INFORMATION

RESTRICTED